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DNA Hybridization To Interface Current-Producing Cells with Electrode Surfaces

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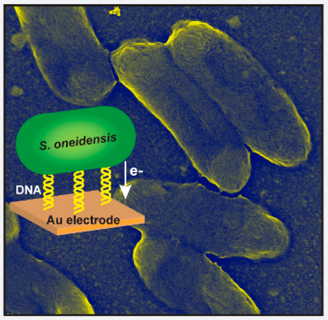
Supporting Information

ABSTRACT: As fossil fuels are increasingly linked to environmental damage, the development of renewable, affordable biological alternative fuels is vital. *Shewanella oneidensis* is often suggested as a potential component of bioelectrochemical cells because of its ability to act as an electron donor to metal surfaces. These microbes remain challenging to implement, though, due to inconsistency in biofilm formation on electrodes and therefore current generation. We have applied DNA hybridization-based cell adhesion to immobilize *S. oneidensis* on electrodes. High levels of current are reproducibly generated from these cell layers following only 30 min of immobilization without the need for the formation of a biofilm. Upon incorporation of DNA mismatches in the microbe immobilization sequence, significant attenuation in current production is observed, suggesting that at least part of the electron transfer to the electrode is DNA-mediated. This method of microbe assembly is rapid, reproducible, and facile for the production of anodes for biofuel cells.



Controlling the interactions between microbes and electroactive surfaces has been a long-standing goal of clean energy technologies.[1,2] Microbial fuel cells, which rely on the native activity of microorganisms to convert chemical energy to electrical energy, have attracted particular recent interest due to their ability to degrade organics in wastewater and produce energy from abundant biomass.[3−5] One organism of particular note is *Shewanella oneidensis*, a facultative anaerobe that is capable of electron transfer to its environment.[6] *S. oneidensis* reduces metal oxides, among other chemical species, for cellular respiration. This exoelectrogen, or organism that can transfer electrons exogenously with its environment in the absence of artificial mediators, can shuttle electrons through several mechanisms. Important electron transfer (ET) pathways for this microorganism include heme-containing proteins in its outer membrane,[7,8] secreted flavins,[9] and the protrusion of biological nanowires.[10−12] Surface-bound *S. oneidensis* cultures produce useful levels of electrical current after the formation of dense biofilms (occurring over several days).[13,14] However, applications of these electroactive biofilms have been limited due to the time required for biofilm formation and the inconsistency in current generation from biofilms grown on electrodes.[15]

Some interesting work has been done to immobilize *S. oneidensis* in the presence of exogenous facilitators of cell−electrode interactions (e.g., carbon nanotubes,[15] gold or palladium nanoparticles,[16] sputtered gold,[17] or silica[18]). Useful currents can be generated using these strategies, but microbe assembly on electrode surfaces remains inconsistent. These devices would benefit from methods that can attach living cells to conductive materials with higher degrees of control and reproducibility, while also requiring less time. In this work, we report the use of a DNA-based cell adhesion method[19] for the efficient and tunable attachment of *S. oneidensis* to electrode surfaces (Figure 1a). In addition to providing a new level of control over the cell density, high levels of current are

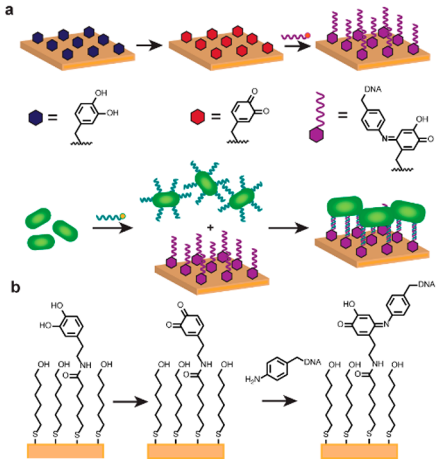


Figure 1. Assembly of *S. oneidensis* layers on gold surface. (a) *S. oneidensis* are added to a gold surface modified with DNA by modifying the cells with complementary DNA. Upon incubation of the DNA-modified microbes with the surfaces, well-defined layers of cells are formed. (b) Mixed monolayers containing catechol termini are first formed on gold electrodes. An applied potential oxidizes the coupling groups, which in turn react with anilines introduced on the DNA strands.

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generated from cells immobilized with this method without requiring the formation of biofilms. The data suggest that the current generated is facilitated by the DNA duplexes that adhere the cells to metal surfaces. This method of microbe assembly thus provides a fast, efficient, and reproducible technique for the fabrication of anodes for biofuel cells.

DNA has unique abilities of self-recognition and electron transport,[20,21] which have led to its broad application in fundamental scientific and medical devices.[22−26] We have previously demonstrated the ability of DNA strands attached to the surface of cells to anchor mammalian, yeast, and bacterial cells to surfaces bearing the sequence complements.[19,27−29] Importantly, for all of these species we can control the density of cells on a surface by controlling the amount of DNA on the underlying solid support.[30] Relative to other adhesion methods, the oligonucleotide-based strategy offers broad applicability, faster immobilization, improved durability, and the potential for substrate reuse. In the case of *S. oneidensis*, synthetic cell surface DNA can be introduced through the oxidation of external glycans with periodate, followed by hydrazone formation with hydrazide-functionalized nucleic acid oligomers. In a previous report we showed that the resulting cells can be attached to glass slides bearing the sequence complement.[29] This provides one of the first available methods for the direct association of these cells to a substrate of interest.

Studies of *S. oneidensis* immobilized on electrodes through DNA hybridization began by using an electrochemical oxidative coupling method for generating electrodes with DNA arrays of varying sequences and densities. Briefly, catechol monolayers were oxidized at 0.3 V versus AgCl/Ag in the presence of aniline-terminated DNA strands (Figure 1b). This led to controllable levels of surface coverage within a few minutes, as quantified using ruthenium hexammine.[30] These DNA-modified surfaces were next exposed to *S. oneidensis* cells bearing complementary sequences. Optically transparent gold electrodes were used to allow fluorescent cell imaging following immobilization. As the amount of DNA on the underlying electrode surface was varied, the total coverage of cells corresponded proportionally to the strand density. A maximum cell coverage was achieved when a 90:10 catechol:mercapto-hexanol ratio was used for gold SAM formation (Supporting Information, Figure S1). We can therefore control the surface density of *S. oneidensis* by varying the density of DNA on the electrode. For the experiments described herein *S. oneidensis* coverages corresponding to 50:50 catechol:mercaptohexanol feedstocks were used to ensure that the cells were at a low density on the electrode and to discourage biofilm formation so as to obtain clear electrochemical data.

Cells were allowed to immobilize on electrodes for 30 min prior to anaerobic incubation. Scanning electron microscopy (SEM) images of the immobilized microbes were acquired 6 h later. Images of the electrode surfaces confirmed that the *S. oneidensis* cells were at a low density on the electrode surface and that the cells maintained a biologically relevant morphology after attachment. At this time point, we observed neither the characteristics of biofilm formation nor the secretion of electroactive nanowires (Figure 2).

We then evaluated the ability of the cells immobilized on these electrodes to produce current. The bound cells were observed to produce significant levels of current compared to buffer alone (Figure 3). Current densities of 39 $\mu A/cm^2$ were observed after 10 h of anaerobic measurement (Figure 4), which compare quite favorably to measurements reported for

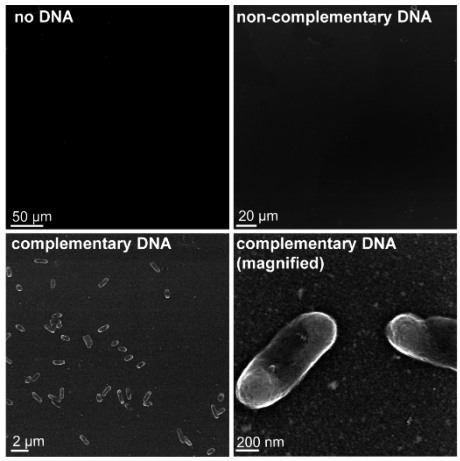

Figure 2. Scanning electron microscopy (SEM) images of *S. oneidensis* layers on electrodes after 6 h of incubation. In the absence of DNA or if a completely noncomplementary sequence is attached to the gold, no microbes adhere. if the DNA is complementary, *S. oneidensis* adhere at low densities with no features characteristic of biofilms. This can be seen upon magnification of the cells. No bacterial nanowires are observed to extend from the cell surfaces.

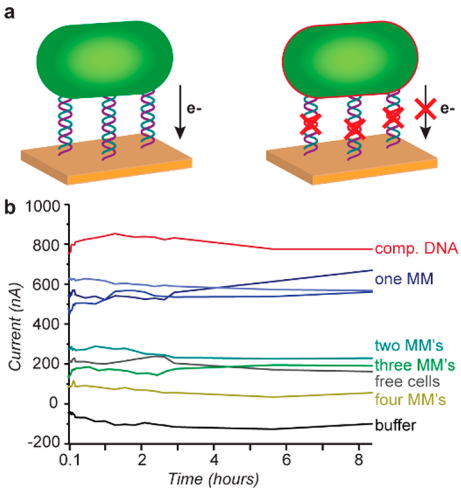

Figure 3. Current output based on DNA sequence. (a) *S. oneidensis* were immobilized on electrodes with either completely complementary (comp.) DNA or DNA that contains variable mismatches (MMs). (b) Current generated at the electrode surface biased to 0.2 V vs AgCl/Ag based on the DNA sequence used to immobilize the microbes. As DNA mismatches are incorporated, attenuation in the generated current is observed.

biofilms and carbon-nanotube-immobilized cells (approximately 20[14] and 10 $\mu A/cm^2$,[15] respectively). It is also important to note that small error bars (2.1%) were observed among different biological replicates, highlighting the reliability of the technique. The highly reproducible currents generated without necessitating the formation of dense *S. oneidensis* biofilms emphasizes the utility of this immobilization technique for the generation of anodes.

Taken together, one can conclude from these observations that the current measured from cells immobilized using DNA hybridization was not transported using conventional mechanisms in dense biofilms, as has been reported in other studies.[9,10] Instead, we hypothesized that DNA-mediated charge transport[21] was at least partially responsible. To explore

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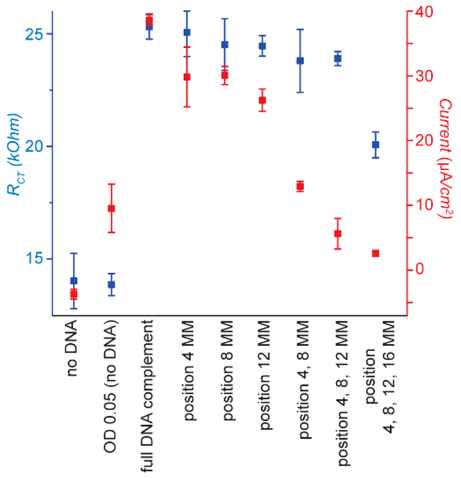

Figure 4. Comparison of relative cell binding measured by electrochemical impedance spectroscopy (blue) to current output (red) as the DNA sequence is varied. All measurements performed after 30 min of cell incubation and 10 h of cells on electrodes. Error bars represent standard error for 3 biological replicates.

this possibility, electrodes were modified with either the perfect complement to the DNA sequence on the cells, or sequences that contained one, two, three, or four nonadjacent base mismatches. Based on calculated melting temperatures, all of these sequences except the sequence containing four mismatches should form duplex DNA at ambient temperature in the applied buffer conditions (Supporting Information, Table S1).

To ensure that equivalent numbers of cells were immobilized on electrodes independent of the number of mismatches incorporated, electrochemical impedance spectroscopy was used to evaluate cell binding. By determining the charge transfer resistance from a solution of ferricyanide/ferrocyanide at *S. oneidensis*-modified electrodes, the relative amount of electrode surface covered by cells could be determined.[30,31] Based on electrochemical impedance measurements, the DNA-modified *S. oneidensis* cells were indeed immobilized with similar densities on DNA-modified electrodes with up to three mismatches (Supporting Information, Table S1 and Figure S2). Lower capture efficiencies were observed with four mismatches, as expected.

As the number of cells bound to the electrode was not found to vary based on the number of mismatches, we then evaluated the currents generated from the different sequences. The current was measured as a function of time by constant potential amperometry. Though 10 h time points were chosen for biological replicates because it was a sufficient length of time to demonstrate consistent current generation without entering the realm of full biofilm formation, long-term experiments conducted with well-matched DNA demonstrated steady current for up to 36 h. Interestingly, the current decreased steadily as mismatches were sequentially incorporated into the DNA strands (Figure 3 and Figure S3). On average, a 23% decrease in current was observed upon incorporation of a single base mismatch, a 61% decrease upon incorporation of two mismatches, a 78% decrease with three mismatches, and finally an 85% decrease upon incorporation of four mismatches. Importantly, when the decrease in current caused by the incorporation of DNA base mismatches was compared to the relative cell binding measured by EIS, it became apparent that the decrease in current was not simply due to the reduced numbers of microbes bound to the electrode (Figure 4). These data lend further support to the hypothesis that DNA plays a role in the transport of electrons between the electrode surface and the *S. oneidensis* cells. DNA likely works in conjunction with previously known mechanisms that shuttle electrons across the periplasmic space.[7−12]

If DNA is indeed participating in electron transfer between the *S. oneidensis* and the electrode surface, then cutting the DNA with restriction enzymes or nucleases should cause a decrease in current. In order to determine the effects of this cutting of the DNA on the observed current, cells assembled with fully complementary DNA were subjected to nuclease or restriction enzyme treatment after allowing the electrodes to run for varying lengths of time. Cell-modified electrodes were run for either 1, 5, or 10 h prior to enzymatic treatment. The *S. oneidensis* cell layers were then exposed to (1) a nonselective nuclease (DNase I), (2) a restriction enzyme with a cut site within the DNA sequence used for immobilization (HinIF), or (3) a restriction enzyme that was not expected to cut the anchoring sequence (EcoRI). The change in current following enzyme treatment indicated that, especially at early times (1 and 5 h), the presence of DNA was essential for the efficient transfer of electrons (Figure 5). As hypothesized for the

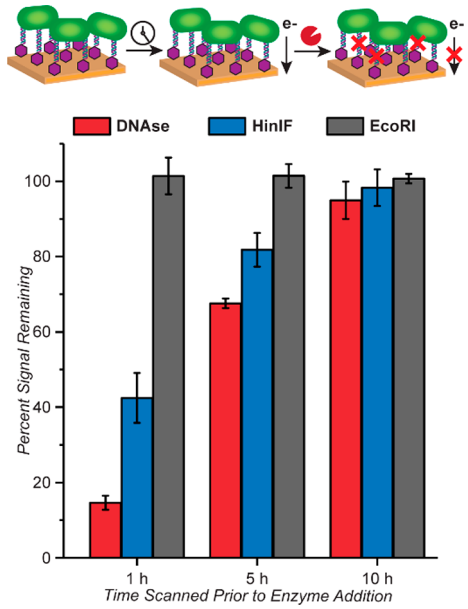

Figure 5. Effect of three enzymes on signal after varying times of *S. oneidensis* current generation. Electrodes modified with the cells are held at 0.2 V vs AgCl/Ag for varying lengths of time, followed by treatment with one of the enzymes. The change in current output is quantified as a percent signal remaining as compared to the original signal. Nuclease (DNase I, red), a restriction enzyme that binds the DNA sequence on the electrode (HinIF, blue), and a restriction enzyme that does not bind the DNA sequence on the electrode (EcoRI, gray) are compared. Error bars represent the error from 3 biological replicates.

participation of DNA in electron transport, the signal from cells on the surface decreased upon addition of the enzymes. The time-dependence of these observations likely results from initial stages of biofilm formation[32,33] as the cells remain on the surface for longer periods. It has been shown that micro-colonies of *S. oneidensis* MR-1 form within 6 h under flow

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conditions.[34,35] The secretion of biofilm components therefore likely contributes to the diminished effects of the enzyme in two ways: (1) the buildup of extrapolymeric substance (EPS) likely renders it more difficult for the enzymes to reach the DNA, and (2) early stages of bacterial nanowire and flavin secretion may also contribute to the maintenance of current at that time.

Taken together, our results indicate a new method of forming electroactive layers of cells on electrodes. This method of immobilization, DNA-based cell adhesion, has enabled the controlled placement of *S. oneidensis* on electrodes and the study of its electron transfer with more control than has been previously available, and with excellent reproducibility. Both consistently high and reproducible currents are currently extremely difficult to generate with *S. oneidensis* due to challenges of reproducible biofilm formation on electrodes. Importantly, we found high levels of current generation from low densities of *S. oneidensis* cells that exhibit no features of biofilm formation or nanowire extension. The data suggest that the DNA used for *S. oneidensis* immobilization is at least partially responsible for the transfer of electrons from the cells to the electrode surface, as decreases in current production are observed upon the incorporation of DNA mismatches and upon enzymatic DNA cleavage. These results provide another compelling reason for using DNA hybridization for cell adhesion. This technique for bioelectrochemical cell construction could be used to elucidate many additional features of electron transfer by dissimilatory metal-reducing organisms.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscentsci.8b00255.

Methods and materials including experimental details, additional electrochemical data, electrochemical setup used, and list of DNA sequences used (PDF)

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Notes

The authors declare no competing financial interest.

Safety Statement: no unexpected or unusually high safety hazards were encountered.

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